Candidate gene for natural immune competence in chickens revealed through genome wide association study

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Summary

Natural antibodies (NAb) are antibodies that are present in individuals without prior exposure to the antigen that they bind. In chickens, NAb have been associated with survival. Furthermore, genetic variation between chickens in NAb levels has been demonstrated. To improve our understanding of the genetic variation for NAb levels in chickens we performed a genome wide association study. A population of 1,628 White Leghorn chickens was investigated. Animals were phenotyped for NAb levels using the antigen keyhole limpet hemocyanin (KLH) as model. Animals were genotyped for 15,579 single nucleotide polymorphisms (SNP). Highly significant association between SNP and NAb levels was observed on chicken chromosome 4. A single SNP was identified as the most likely candidate for this association. Identification of this candidate SNP was based on further analysis with full genome sequence genotypes and on predicted consequences of associated SNP. This candidate SNP causes a phenylalanine to leucine amino acid change in one of the leucine rich repeats in toll-like receptor 1 family member A (TLR1A). Our results suggest an important role for TLR1A in relation to NAb levels in chickens. Further study should confirm the effect of the SNP in *TLR1A* on NAb levels and on the (natural) immune competence of chickens.

Keywords: natural antibodies, toll-like receptor, TLR1A

Introduction

Natural antibodies (NAb) are antigen-binding antibodies that are present in individuals without prior exposure to the antigen that they bind. Natural antibodies play an essential role in the innate and adaptive immune defense and, thus, contribute to (general) disease resistance. Different NAb isotypes are found: predominantly IgM, but also IgA and IgG.

Natural antibodies binding the antigen keyhole limpet hemocyanin (KLH) have been used as model. Keyhole limpet hemocyanin is a protein found in Californian sea snails, and chickens have probably not encountered nor will encounter KLH during life. High levels of NAb binding KLH have been associated with survival in laying hens (Star et al., 2007; Sun et al., 2011). Furthermore, levels of NAb binding KLH in chickens have been shown to be heritable (Sun et al., 2013; Berghof et al., 2015). Thus, selective breeding for NAb levels might be an effective strategy to improve the chickens' immune competence and,

subsequently, their performance in practical production systems.

The aim of this study was to improve our understanding of the genetic variation for NAb levels in chickens. We screened the chicken genome for associations with NAb levels, in order to find genomic regions or genes that are related to NAb levels. Such knowledge will help to understand the role of NAb in the (natural) immune competence of chickens.

Material and Methods

Population

Samples and data were obtained from a purebred White Leghorn chicken line that was housed on a breeding nucleus farm of Institut de Sélection d'Animale (ISA) in the Netherlands. The population comprised 1,628 animals (696 males and 932 females), and descended from 112 sires and 288 dams. Blood was collected once between 15 and 22 weeks of age. Blood samples were centrifuged to separate plasma and cells. Both blood fractions were stored at −20°C until use for phenotyping and genotyping.

Phenotypes

Blood plasma was used to determine NAb levels binding the antigen keyhole limpet hemocyanin (KLH). Total KLH-binding antibodies (IgT) as well as the isotypes IgM, IgA and IgG were quantified by indirect two-step ELISA as described by Berghof et al. (2015).

Genotypes

Blood cells were used for extraction of DNA and subsequent genotyping for 2,740 single nucleotide polymorphisms (SNP; 488 animals) or 11,173 SNP (1,140 animals). Genotypes were imputed to a set of 57,636 SNP genotypes, based on approximately 120 key ancestors of this chicken line. After removing 37,053 non-segregating SNP and quality control, 15,579 SNP genotypes were available for genome wide association analysis. Significantly associated regions were imputed to full genome sequence genotypes, based on 70 key ancestors. Imputation was done based on Gallus_gallus-5.0 using Beagle 4.0 (Browning & Browning, 2007).

Association Analysis

Single SNP association was analysed with the following animal model:

$$Y_{ijk} = \mu + P_i + \beta_1 * Age_{ijk} + SNP_j + id_k + e_{ijk} \quad (1)$$

Where Y_{ijk} is the NAb titer; μ is the overall mean; P_i is the fixed effect of plate on which the NAb titer was quantified (i = 1-91 for IgT, IgM and IgG NAb, i = 1-100 for IgA NAb); Age_{ijk} is the covariate describing the effect of age of the animal when blood was collected (in weeks); SNP_j is the fixed effect of SNP genotype class (j = AA, AB or BB); id_k is the random additive genetic effect of animal assumed to be distributed as $\sim N(0,\mathbf{A}\sigma_a^2)$ with additive genetic relationship matrix **A** and additive genetic variance σ_a^2 (the pedigree consisted of 2,537 animals with at least 3 generations of ancestors); and e_{ijk} is the random residual effect assumed to be distributed as $\sim N(0,\mathbf{I}\sigma_e^2)$ with identity matrix **I** and residual variance e_{ijk}. For IgM NAb, the model was extended with a random dam effect assumed to be distributed as $\sim N(0,\mathbf{I}\sigma_m^2)$ with identity matrix **I** and maternal environmental variance σ_m^2. Association analysis was performed using ASReml 4.1 (Gilmour et al., 2014).

Possible consequences of nucleotide changes of associated SNP were predicted using

SIFT (Sim et al., 2012) and PolyPhen-2 (Adzhubei et al., 2010).

Results & Discussion

Genome wide association analysis for levels of NAb binding KLH was performed using 15,579 SNP genotypes for 1,628 White Leghorn chickens. No associations were detected for levels of IgG NAb. Suggestive association (false discovery rate (FDR) ≤ 0.20) was found for levels of IgT NAb on chromosome 4 (3 SNP), and for levels of IgA NAb on chromosome 9 (2 SNP) and on chromosome 18 (3 SNP). Significant association (FDR ≤ 0.05) was detected for levels of IgM NAb on chromosome 4 (35 SNP; Figure 1).

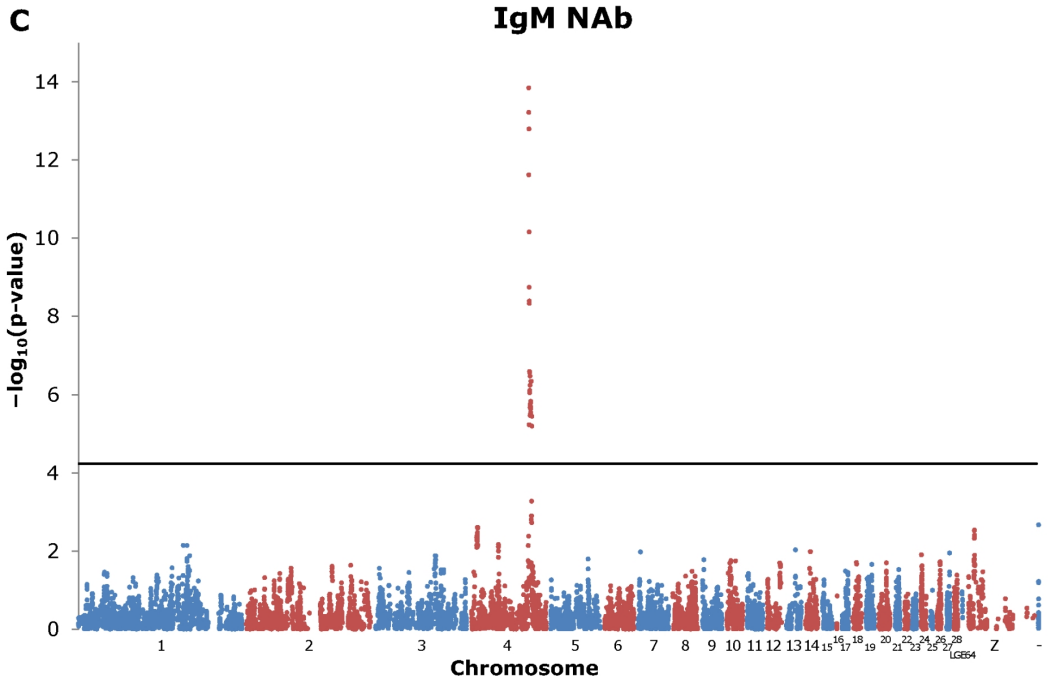

Figure 1. Significance (−log$_{10}$(p-values)) of associations between 15,579 genome wide SNP genotypes and levels of KLH-binding IgM NAb measured in plasma of 1,628 White Leghorn chickens.

Association analysis with full genome sequence genotypes confirmed the most significant associations for IgM NAb levels on chromosome 4 between 69.5 and 70.0 mega base pairs. This region contains 15 genes. Based on predicted consequences of nucleotide changes of all SNP in the associated region, one SNP (at 69,965,939 bp) was identified as the most likely candidate for the observed association with IgM NAb levels. This SNP causes a phenylalanine to leucine amino acid change in one of the leucine rich repeats (at position 126) in toll-like receptor 1 family member A (TLR1A).The association of this SNP with IgM NAb levels had a −log$_{10}$(p-value) of 15.11 and accounted for approximately 63.5% of the additive genetic variance.

The results suggest an important role for TLR1A in relation to NAb levels in chickens. TLR1A is one of 10 known chicken toll-like receptors. Toll-like receptors are transmembrane proteins that recognize conserved molecular motifs from a broad range of pathogens.

Interaction of TLR with such motifs results in initiation of both innate and adaptive immune responses (Brownlie & Allan, 2011). The position of the SNP, resulting in an amino acid change in leucine rich repeat 4 of TLR1A, suggests that it does probably not affect ligand recognition (Keestra et al., 2007). Instead, the effect of the SNP could result from consequences of its impact on the structure of the protein.

The SNP in *TLR1A* showed full dominance: one of the homozygous genotype classes had similar IgM NAb levels as the heterozygous genotype class, while IgM NAb levels of the other homozygous genotype class were significantly lower. This suggests that one regular copy of *TLR1A* is sufficient for obtaining normal NAb levels.

Further research must confirm the effect of the SNP in *TLR1A* on NAb levels and on the (natural) immune competence of chickens.

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